1,015,284

PATENT SPECIFICATION

NO DRAWINGS

1,015,284

Date of Application and filing Complete Specification: Sept. 21, 1962.
No. 36007/62.
Application made in United States of America (No. 141,565) on Sept. 21, 1961.
(Patent of Addition to No. 900,181 dated Nov. 28, 1958.)
Complete Specification Published: Dec. 31, 1965.

Index at acceptance:—C3 H2
Int. Cl.:—C 07 g

COMPLETE SPECIFICATION

Preparation of Collagenous Materials

We, ETHICON, INC., a corporation of the State of New Jersey, United States of America, of Somerville, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to polymerization products of fibrous proteins. More particularly, the present invention is concerned with dye-photocatalyzed collagenous polymers and methods for their preparation and is an improvement in or modification of the invention described and claimed in Patent Specification No. 900,181.

Collagen, a proteinaceous substance present in the white connective tissue of animals, has long been employed as raw material for the preparation of leathers and surgically useful articles such as sponges, films, fibres, filaments, etc. The conversion of collagen into utilitarian forms requires a tanning step wherein an agent combines with collagen, more or less reversibly, transforming the collagen into a fixed form. Tanning agents which are commonly used in the collagen field include formaldehyde, glyoxal, chrome and vegetable tannins. All of the known tanning agents have one common property with respect to their tanning ability on collagen—they not only participate in the tanning reaction, but also become an integral part of the final tanned product. Chrome, for instance, may be a constituent of the final product to the tenure of up to 5% or more; vegetable tannin of up to 50% or more; formaldehyde of up to several per cent; oil tannins of up to 10%. Conventional tanning agents combine with various active groups of the peptide chain such as carboxyl, amino and hydroxyl groups to form bridges between or along these chains anchored by various types of bonds, including hydrogen, covalent and salt bonds.

Among the methods currently employed for converting collagen into useful articles is the process which comprises preparing an aqueous acid dispersion of collagen tissue and precipitating the collagen by the addition of alkali such as ammonium hydroxide. The precipitated collagen is then converted to the desired article by appropriate dehydration procedures, as for example, by evaporation of water to prepare a film, or by spinning to prepare a fibre or filament. Tanning agents are incorporated during or after formation of the article.

Articles prepared according to the above-described process are based on the conversion of collagen to a dispersion wherein the collagen is in a fibrillar state rather than a molecular dispersion and attraction between fibrils is limited to weak and easily ruptured forces such as van der Waals forces and hydrogen bonds. Articles prepared from such dispersions have a relatively low break strength. Essentially, therefore, the methods heretofore employed are predicated on the breaking of collagen tissue with mild acid media and its subsequent reconstitution into newly-formed bundles of fibrils by means of alkali or dehydrating agents, usually followed by treatment for stabilization with agents such as the previously mentioned tanning agents.

In the Patent Specification No. 900,181 (the Parent Specification) there is described and claimed a photosensitive isoalloxazine-catalyzed, irreversibly cross-linked, thermostable polymer of collagenous material and a method of making same.

It has now been discovered that novel, thermostable, irreversibly cross-linked collagenous polymers can be prepared by irradiating collagenous substances such as collagen, *per se*, derivatives of collagen and collagen degradation products with visible and/or ultraviolet light in the presence of a dye other than as isoalloxazine. The novel polymers are distinguishable from known tanned collageonus products in that the dye is employed solely as a catalyst to induce cross-bonding reactions between amino acids of adjacent collagen

chains, and, therefore, does not become chemically integrated with the final product. Thus, collagenous polymers are obtained which are structurally free from the dye which photocatalyzed their formation.

Although the mechanism of polymer formation cannot be postulated with certainty, the dye is believed to photocatalyze the formation of a charge transfer complex between the photocatalyst and two amino acids of adjacent protein chains, to give a high order of cross-linking resulting in the formation of novel collagenous polymers. The dye then extracts electrons from the amino acids, leaving them bound together by a covalent bond. Experiments indicate that films, filaments, fibres and sponges made from collagenous starting materials cross-linked in this manner possess properties characteristically different from articles formed from collagenous starting materials not so treated. Such cross-linked products have a higher tensile strength, lower water retention, less shrinkage on heating in water at 100°C., and less swelling in water than similar bodies formed without such treatment. Stress-strain analyses, viscosity and syneresis measurements indicate that the cross-linking is such that the collagenous polymers have increased thermostability and are much greater in size than their precursors.

Although physical-chemical properties similar to those of the present products may be obtained by processes of the prior art generally known as "tanning", the final cross-linked collagenous polymers resulting from the present invention are (in contrast to the prior art) entirely free from the dye photocatalyst which effected the polymerization. In this respect, the novel polymer differs from tanned collagenous products, i.e., the latter contain, as an integral part of the final structure, substantial quantities of the tanning agents used to promote the reaction. The freedom of the novel polymers from the cross-linking agent is considered of particular importance in the light of biological considerations, i.e., its use in the fields of medicine, surgery, or for food purposes.

According to the present invention, the conversion of native collagen to the novel polymeric thermostable products is accomplished by the exposure of these starting materials to irradiation with visible and/or ultra-violet light, either artificial or natural, in the presence of a dye other than an isoalloxazine. Since this fundamental discovery embraces a wide variety of permutations and modifications, it is intended that these be included within the scope of the present invention.

In one of its embodiments, the novel process comprises, in a general way, the preparation of a collagenous gel in which the collagenous substance, i.e., the collagen derivative, degraded collagen or, preferably, collagen is converted to a polymerized, cross-linked form by exposing an acid dispersion of the collagenous starting materials to irradiation in the presence of a photosensitive dye other than isoalloxazine. The gel of polymerized collagenous material is then shaped to the desired form, e.g., by evaporation, neutralization or dehydration. The cross-linked gel is so bonded that articles made from it possess distinctively high tensile strength and rapid *in vivo* absorption time, as well as rapid in vitro digestion rate. More specifically, films and fibres made from such gel are characterized by high break strength, low swelling in water, low shrink temperature and rapid degradation by proteolytic enzymes. Sponges prepared from such gel are characterized by being not readily redispersible in water and being rapidly absorbable *in vivo*.

In accordance with the preferred embodiment of this invention, the acid dispersion is shaped and reconstituted to the desired form, i.e., by neutralization or dehydration, and the reconstituted collagenous product *in situ* or rewetted with water if previously dried, is subjected to the dye photocatalysis reaction. In other words, irradiation in the presence of the dye may accompany or follow the neutralization, and complete or incomplete dehydration, and/or evaporation.

The term "collagenous" material as applied in accordance with this invention to the starting materials and to the corresponding polymeric final products is intended to embrace collagen itself, that is to say, the protein commonly known as forming the chief constituent of connective tissue and the organic substance of bones, as well as collagen derivatives, collagen degradation products and collagen extracts.

One may therefore employ as a starting material collagen in any of its forms, i.e., native, fibrous collagen of tendon, hide, gut, fish swim bladder (ichthiocoll); dissolved collagen in the molecular state; dispersed collagen in the fibrillar state or collagen reconstituted to the solid fibrous state from molecular or fibrillar states. In practice, the native or reconstituted collagen is immersed in, or the dispersed or dissolved collagen is added to, as the case may be, a solution of a dye and the preparation is irradiated under conditions insuring adequate light penetration, after which shaped articles are formed. Such articles are conveniently made from the resulting polymer according to methods known to those skilled in the art and generally discussed herein.

The term "collagen degradation product" as used in this invention is intended to embrace partially degraded collagen, such as, for example, gelatin obtained from collagen, preferably of high molecular weight with Bloom of 205 or greater.

Collagen derivatives such as native collagen whose available side groups have been esterified by alcoholysis, are also suitable collagenous

starting materials for the preparation of the novel polymers of this invention. The esterification is suitably accomplished with an alkanol having from about 1 to about 20 carbon atoms, preferably a lower alkanol such as methanol, ethanol, propanol, butanol, pentanol, hexanol, iso-pentanol, iso-hexanol, etc. in the presence of a dilute inorganic acid such as sulphuric, hydrochloric, phosphoric, etc. The esterified collagen so obtained is then dispersed in aqueous solution, and subjected to irradiation in the presence of a dye. The resulting gel is reconstituted by evaporation to the desired form. Alternatively, the esterified collagen may be directly reconstituted and the resulting product then subjected to the irradiation process.

Because the photocatalytic reaction of this invention induces a charge transfer mechanism within the collagenous starting materials, it is possible, by means of this reaction, to introduce a wide variety of moieties into the starting materials simultaneously, *in situ*, during their photocatalytic transition into collagenous polymers. The charge transfer is induced by the dye photocatalyst which is capable of utilizing light energy to remove electrons from a large number of organic substances, thus creating covalent bonds suitable either for polymerization of the collagenous starting material itself, or formation of copolymers of collagenous materials with other substances or, as is usually the case, both.

An extremely wide variety of substances may be used for participating in the charge transfer reaction of this invention, the only limitation on the character of the material to be employed being that it should have atoms or radicals capable of donating electrons to the light activated dye. Among substances which are suitable for this purpose, are those having one or more of the following groups within their structure; amino, hydroxyl, guanidyl, amido, imido, imino, sulphydryl, ether linkages, aldehydo, keto and functional derivatives thereof. More specifically, one may employ for this purpose amino acids such as glycine, alanine, serine, theonine, valine, leucine, isoleucine norleucine, phenylalanine, tyrosine, cysteine, cystine, methionine, tryptophane, proline, hydroxyproline, aspartic acid, glutamic acid, histidine, arginine, lysine, hydroxylysine and citrulline; nucleic acids and nucleoproteins; carbohydrates, including monosaccharides, e.g., hexoses such as glucose, fructose, galactose and mannose; pentoses such as arabinose, xylose, ribose, rhamnose, and desoxyribose; disaccharides such as maltose, lactose, sucrose, gentobiose, isomaltose and cellobiose; trisaccharides such as raffinose; and polysaccharides such as starch, inulin, glycogen, dextrin, cellulose and pectin.

As used in this invention, "dyes" are chromophore-containing compounds other than isoalloxazines capable of complexing with collagenous materials. A wide variety of substances possessing these properties may be employed in accordance with this invention, provided only that they are capable of utilizing light energy to remove electrons from organic substances as specified above. Obviously, inclusion of a chromophore group in the structure is essential if light absorption is to be effected. The feature of complexation is one which can be readily ascertained by simple experiment.

Although it is preferred to use a dye which forms a weak, readily reversible complex with collagenous materials (in order to facilitate easy removal, if so desired), dyes which form relatively strong and stable complexes may be employed in cases where retention of a colour in the final product is unobjectionable or desirable.

Chromophore-containing substances capable of complexing with collagenous materials, which may be employed include those falling within, but not necessarily limited to, the following groups: *tetrazoliums* such as 2,3,5-triphenyltetrazolium chloride; *pteridines* such as 2,4-(1H,3H)-pteridinedione (lumazine); *pterines* such as xanthopterin; *azo* such as 4,4^1 - bis - [7 - (1 - amino - 8 - hydroxy - 2,4 - disulpho) - naphthylazo) - 3,3^1 - bitolyl tetra-sodium salt (Evans Blue); *azines* such as aminodimethylaminotoluaminazine hydrochloride (neutral red); 3,7-diamino-5-phenylphenazinium chloride (phenosafranin); and tetraethyl phenosaphranin (amethyst violet); *thiazines* such as 2-amino-7-dimethylamino-1(or 3)-methylphenazathionium chloride (toluidine blue O); 3 - methylamino - 7 - dimethylaminophenazathionium chloride (Azure B); 3 - amino - 7 - dimethylaminophenazathionium chloride (Azure A); 2,7 - diaminophenazathionium chloride (thionine); 8-nitro-2,7-bis-dimethylaminophenazathionium chloride (methylene green); 3,7-bis-(dimethylamino) phenazathionium chloride (methylene blue); *indamines* such as Bindschedler's Green; *oxazines* such as 7-oxy-phenozazon-(2)-10-oxide (resazurin); *acridines* such as 3,6-diaminoacridinium chloride hydrochloride (proflavine); 2,7-dimethyl-3,6-diaminoacridinium chloride hydrochloride (acridine yellow); 3,6 - bis - (dimethylaminoacridinium chloride hydrochloride (acridine orange); *triphenylmethanes* such as zinc oxalate or ferric double chloride of tetramethyl para-aminotriphenylcarbinol (Malachite green); *fluoranes* such as 9 - (o-carboxyphenyl) - 6 - hydroxyl - 3 - isozathenone (fluorescein); the sodium or potassium salt of tetra-iodofluorescein (erythrosine); the sodium or potassium salt of tetra-bromofluorescein (eosin Y); 9 - (tetrachloro - o - carboxy - phenyl) - 6 - hydroxy - 3 - isozathenone (rose bengal); *pyronines* such as 3,6 - bis - (methylamino) - pyroninium chloride hydrochloride (acridine

red 3B); and *thiazoles* such as 2-(p-aminophenyl) - 6 - (6¹ - methylbenzothiazole)-benzothiazole (primulin).

Depending upon the starting material employed, the hydrogen ion concentration during irradiation may be varied widely from a pH of about 2.0 to about 14.0. In the case of an acid dispersion, desirable results are obtained when the irradiation is carried out under acid conditions; that is to say in the pH range from about 2.0 to about 4.5, preferably from about 3.0 to about 4.5. If the reconstituted form of collagen is employed, the pH may be on the alkaline side, i.e. from about 5.0 to about 14.0, preferably from about 9.0 to about 11.0.

The photocatalysis proceeds smoothly with relatively small quantities of catalyst as well as with larger quantities. Therefore, the only limitation as to the amount of dye which may be employed will be that circumscribed by the solubility of the particular compound used. Experience indicates that quantities of dye ranging from about 0.2 mg. to about 50 mg. for each 100 grams of dispersion are satisfactory, and that optimal results are obtained with quantities of about 50 mg. of dye for each 100 grams of dispersion. In the case of reconstituted or native collagen one may employ from about 1 mg. per cent to 100 mg. per cent, optimally about 5 mg. per cent, dye in the immersion bath.

The time requirement for ensuring completion of the reaction with a given concentration of photocatalyst varies considerably depending upon the wave length and intensity of irradiation, as well as the purpose for which the polymer is intended to be used. In general, however, it has been found that suitable polymers are obtained with as little as two seconds exposure to irradiation and as much as several days exposure, depending upon the concentration of photocatalyst, the pH and the state of the collagenous material, as well as the intensity and wave length of light. In practice, a satisfactory period of time for polymerization to take place is from a few, e.g. two or three, seconds up to several, e.g. forty-eight, hours although this is not be be construed as an absolute limiting factor. Variations in light intensity and time appear unlimited except for factors having detrimental effects on the collagenous material.

The most effective wave length of light is in that range of the electromagnetic scale which is maximally absorbed by the photocatalyst, i.e. the ultaviolet and visible light regions represented in the range of from about 2500 to about 7000 Angstrom units. Optimal results are obtained at wave lengths most strongly absorbed by the given dye. The source of visible light may be either natural, i.e. sunlight, or artificial, i.e. emanating from an arc lamp, fluorescent bulb or similar device. The process is operative at a light intensity varying from about 10 foot candle power to about 13,000 foot candle power, the latter being equivalent to the intensity of sunlight. A general rule which may be followed is that the intensity employed is directly proportional to the time required for the polymerization reaction to reach completion. Thus, the higher the intensity of the light, the shorter will be the time requirement, and vica versa. If the operation is carried out at a relatively intensive range of foot candle power, e.g. sunlight for a protracted period of time, precautions must be taken to keep the reaction mixture at a temperature sufficiently low to preclude denaturation of the collagenous material and change in its configuration. Since denaturation of dispersed collagen takes place usually at about 37°C., temperatures in excess of this are preferably not employed with dispersed collagen, although a temperature of about 40°C. maximum may be used in cases where exposure to irradiation is only for a brief period of time. If reconstituted or native collagen is used, temperatures below 60°C. are preferably employed.

The concentration of collagenous material in the acid dispersion or aqueous solution employed as starting material for forming reconstituted collagenous polymers may be varied over wide limits, in some cases from 0.01 grams of collagenous substance per 100 ccs. up to about 2 to 5 grams per 100 ccs, but in others considerably higher. For example, it may be desirable in some cases to prepare a relatively thick dispersion, i.e. where the polymer is intended for use in the preparation of a film. Under such circumstances, the operation may be carried out in media having collagenous concentration of the order of 80 to 95 grams per 100 ccs. This may be accomplished by first dissolving the photocatalyst in the acid dispersion or solution, concentrating such dispersion or solution by a suitable method, e.g. evaporation, and then subjects the concentrate to irradiation. In actual practice a convenient concentration suitable for most purposes is from about 0.2 to about 5 grams per 100 ccs. Since it is necessary to have the photosensitive catalyst completely dispersed in the medium while at the same time preventing cross-linking until the desired stage of dispersion, shaping or reconstitution is reached, it is preferable to carry out the preliminary operations under subdued light.

The preparation of the collagenous dispersion may be carried out in accordance with the methods well known to those skilled in the art. For example, the raw collagen material previously washed clean of debris, is added to an aqueous acid solution and allowed to soak until it is swollen, the swelling taking place because of the breaking of the hydrogen bonds by the acid. Suitable acids which may be employed for this purpose are: sulphuric, hydrochloric, acetic, citric, lactic, etc. in a concentration from about 0.001N to about 0.1N. If

desired, an enzyme system such as commercial malt-diastase or ficin may be used to treat the collagen preliminarily to break up elastin and carbohydrate compounds.

As neutralizing agents for reconstitution there may be used organic or inorganic bases such as tertiary amines, alkali metal and alkali earth metal hydroxides, alcoholates, hydrides, amides or hydrocarbon compounds of alkali metals or alkali earth metals. As specific examples there may be included sodium hydroxide, potassium hydroxide, pyridine and, preferably, ammonia.

Suitable dehydrating agents for reconstitution of the gel include inert water-miscible organic solvents as, for example lower alkanols and ketones, and tertiary amines such as methanol, ethanol, propanol, butanol, pentanol, isopropanol, dioxane, pyridine, methyl-ethyl-ketone, dimethyl-ketone, diethyl-ketone, etc.

Reconstitution of the gel by evaporation is readily accomplished at room temperature, e.g. 20°C. to 30°C. Slightly higher temperatures, i.e. about 30°C. to 40°C., may be used, if caution is exercised to prevent degradation.

The novel dye-photocatalyzed polymers prepared in accordance with this invention may be converted to a variety of useful articles. For example, the polymer may be lyophilized, i.e. vacuum-dried, from the frozen state to form a sponge which is not redispersible in water and which is rapidly (15 to 25 days) absorbable *in vivo*. Sponges prepared in this manner from the novel polymeric gel may be used for implantation in the body for packing cavities or for hemostatis, or as matrices for tissue culture. If desired, the sponges can be impregnated with drugs such as hemostatic agents, e.g. heparin; hormones, antibiotics, e.g. penicillin, tetracyclines; or wound-healing accelerators, e.g. Vitamin V, Vitamin A, urea or strepogenins, prior to implantation in the body.

The novel dye-photocatalyzed polymers may be used in the preparation of film for surgical use. To accomplish this, the dispersion containing the dye is spread out in a suitably thick layer in a dish or pan or on a screen, irradiated, and the water allowed to evaporate. Films prepared in this manner show very different characteristics from control films, i.e. those prepared by conventional methods directly from collagen dispersions. Among the unique properties there may be cited: increase in break strength; decreased swelling in water; low shrink temperature; and rapid degradation by proteolytic enzymes.

The novel dye-photocatalyzed polymers may be formed at the time of reconstitution by neutralization of the dispersion or solution. To accomplish this the dispersion or solution containing the dye is exposed to the neutralizing or dehydrating agent and simultaneously irradiated. Conversely, the dye is incorporated into the neutralizing or dehydrating bath and irradiation accompanies application of the neutralizing bath. Sponges, films and fibres may be formed by this method.

The novel dye-photocatalyzed polymers may be formed from previously reconstituted collagenous material. The latter is reconstituted from an acid dispersion or a solution by neutralization and/or dehydration. The reconstituted collagenous material is then irradiated in the presence of dye. Collagenous sponges, films and fibres may be polymerized by this procedure.

It may be stated here that the dye-photocatalyzed polymers of this invention show characteristic behavior in gelation and syneresis tests which provide a basis for distinction from conventional aqueous acid collagenous dispersions. For example, when an acetic acid dispersion of collagen containing a photosensitive dye is exposed to visible light, a rapid partial setting to the gel state occurs. Continued exposure results in greater firmness. On the other hand, control samples without photosensitive dye in the light or with photosensitve dye in the dark remain in a liquid state. This simple comparison clearly establishes the necessity for both a photosensitive catalyst and light. The syneresis phenomenon is manifested when collagenous dispersions are gradually heated. Dispersions containing no photosensitive dye undergo a marked lowering of viscosity to a watery state. Conversely, when collagenous dispersions containing a photosensitive dye are heated, the effect is reflected by the time and intensity of previous irradiation. Nonirradiated samples show liquefaction. Irradiated samples exhibit syneresis with marked separation into a gel phase, which remains set at elevated temperatures as well as at room temperature, and an aqueous phase. The relationship between the degree and permanence of syneresis and the time or intensity of irradiation is quantitative. As used herein, the degree of syneresis is expressed in terms of the per cent of liquid expelled from the contacting gel.

The following Example illustrates the invention.

EXAMPLE

A dispersion containing 0.3% collagen is prepared by treating tendon slices with 0.02% ficin, inactivating the ficin with 0.1% hydrogen peroxide and swelling the slices in 0.01M acetic acid, clarifying by centrifugation, precipitating in acetone and redispersing to form a 0.9% dispersion in 0.1M acetic acid. The dispersion is extruded through a hypodermic syringe into acetone. The resulting fibres are dried, placed in phosphate buffer pH 9.0 containing a predetermined concentration of a dye. The preparation is irradiated for sixteen hours at 200 foot candle power. The degree of cross-linking is determined by stress-strain analysis as adapted to collagen by Wiederhorn et al. *J. Am. Leather Chemists, Vol. 48, pp 7—20, 1953*. This technique determines the molecular

weight between cross-links of polymeric materials. The lower the molecular weight between cross-links, the greater the degree of cross-linking. The results given in the Table below are expressed in terms of molecular weight between cross-links.

Dye	Concentration (mg. per cent)	Molecular Weight Between Cross-links	Average Number of Cross-links/ Tropocollagen Unit of 360,000 m.w.
Control (no dye)	—	21,400	16.9
Pheno-safranin	5.0	11,600	31.1
Rose bengal	5.0	11,500	31.2
Methylene blue	5.0	10,300	35.0
Azure A	5.0	10,200	35.3
Toluidine blue O	5.0	11,100	32.6
Eosin Y	5.0	13,200	27.2
Control (no dye)	—	30,800	11.8
Evans Blue	5.0	17,900	20.2
Methylene green	5.0	16,900	21.4
Amethyst violet	5.0	11,700	30.8
Control (no dye)	—	67,000	5.4
Lumazine	5.0	27,000	13.4
Thionine	5.0	5,600	65.0
Control (no dye)	—	17,500	20.6
Xanthopterin	10.0	10,800	33.3
2,3,5-triphenyl-tetrazolium Cl.	1.0	9,500	38.0
Acridine red	1.0	9,600	37.8
Acridine orange	1.0	6,800	53.0
Proflavine	1.0	7,400	48.8
Resazurin	1.0	6,700	53.7
Azure B	1.0	7,000	51.0
Bindschedler's green	10.0	9,600	37.8
Primuline	1.0	8,300	44.0
Acridine yellow	10.0	7,500	48.0
Control	—	41,100	8.7
Neutral red	5.0	11,700	30.7
Erythrosine	5.0	10,100	35.5
Fluorescein	5.0	19,200	18.7
Malachite green	5.0	28,600	12.6

WHAT WE CLAIM IS:—

1. An irreversibly cross-linked, thermostable polymer of collagenous material which has been photocatalyzed with a dye other than an isoalloxazine by irradiation with visible and/or ultra-violet light.

2. A polymer according to claim 1 wherein the dye is any one of those hereinbefore specifically mentioned.

3. A polymer according to claim 1 or 2 in the form of a gel, a sponge, a film, a fibre, or a filament.

4. A method for preparing an irreversibly cross-linked, thermostable polymer of collagenous material which comprises the step of irradiating a collagenous material with visible and/or ultra-violet light in the presence of a photosensitive dye photocatalyst other than an isoalloxazine catalyst.

5. A method according to claim 4 wherein the dye is any of those hereinbefore specifically mentioned.

6. A method according to claim 4 or 5 wherein the collagenous material to be irradiated is in the form of an acid dispersion.

7. A method according to claim 4 or 5 wherein the collagenous material to be irradiated is in the form of an aqueous solution.

8. A method according to claim 7 wherein the collagenous material is in the form of an aqueous salt solution extract.

9. A method according to claim 6, 7 or 8

wherein the concentration of collagenous material in the dispersion or solution is between 0.2 and 5 grams per 100 ccs.

10. A method according to any of claims 4—9 wherein the collagenous material to be irradiated is esterified collagen, reconstituted collagen, native collagen or partially degraded collagen.

11. A method according to claim 4 or 5 wherein the dye is dissolved in an aqueous acid dispersion of the collagenous material, the dispersion is subjected to irradiation, and the resulting gel reconstituted by neutralization.

12. A method according to claim 4 or 5 in which an aqueous acid dispersion of the collagenous material is reconstituted by neutralization, and subjected to irradiation in the presence of the dye.

13. A method according to claim 4 or 5 in which the dye is dissolved in an aqueous acid dispersion of the collagenous material, which dispersion is subjected to irradiation, the resulting gel being reconstituted by dehydration.

14. A method according to claim 4 or 5 in which an aqueous acid dispersion of the collagenous material is reconstituted by dehydration and the resulting product is subjected to irradiation in the presence of the dye.

15. A method according to claim 4 or 5 in which the dye is dissolved in an aqueous acid dispersion of esterified collagenous material, which dispersion is subjected to irradiation, and the resulting gel reconstituted by evaporation.

16. A method according to claim 4 or 5 in which the dye is dissolved in an aqueous solution of esterified collagenous material, the solution is subjected to irradiation, and the resulting gel reconstituted by evaporation.

17. A method according to claim 4 or 5 in which an aqueous acid dispersion of esterified collagenous material is reconstituted by evaporation or dehydration, and the resulting product subjected to irradiation in the presence of the dye.

18. A method according to claim 4 or 5 in which an aqueous solution of esterified collagenous material is reconstituted by evaporation, and the resulting product subjected to irradiation in the presence of the dye.

19. A method according to claim 4 or 5 in which the dye is dissolved in an aqueous salt extract of the collagenous material, the extract is subjected to irradiation, and the resulting gel reconstituted by evaporation.

20. A method according to claim 4 or 5 in which the dye is dissolved in an aqueous salt extract of the collagenous material, the extract is subjected to irradiation, and the resulting gel reconstituted by dehydration.

21. A method according to any of claims 4—20 wherein the irradiation is carried out under natural light.

22. A method according to any of claims 4—20 wherein the irradiation is carried out under natural light.

23. A method according to claim 21 or 22 wherin the irradiation is carried out at an intensity substantially within the range 10 to 13,000 foot candle power.

24. A method according to any of claims 5—23 wherein the irradiation is carried out at a wave length substantially within the range 2500 to 6000 Angstrom units.

25. A method of preparing an irreversibly cross-linked thermostable polymer of collagenous material substantially as hereinbefore described otherwise than with reference to the prior art.

26. A method of preparing an irreversibly cross-linked thermostable polymer of collagenous material substantially as described in the foregoing Example.

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Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press (Leamington) Ltd.—1965. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2, from which copies may be obtained.